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# Toxicity of Decolorized Batik Wastewater on Common Carp Using Combination of Chitosan and Spent Mushroom Substrate

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## ABSTRACT

Decolorization is a key treatment process in batik industry wastewater management, aimed at reducing color intensity. This study evaluates a decolorizing combination of chitosan from *Litopennaeus vannamei* (CLV) and spent mushroom substrate from *Pleurotus ostreatus* (SMSPO), examining its effect on the 96-hour $LC_{50}$ value and scale structure of common carp. Using a completely randomized design, common carp was exposed to decolorized wastewater at concentrations of 0%, 1.875%, 3.75%, 7.5%, 15%, and 30%. Results show that decolorized wastewater at 7.5% concentration remains lethal to fish, while concentrations of 3.75% and below are non-lethal. Microscopic changes in fish scales indicate particle adhesion from the wastewater. The 96-hour $LC_{50}$ for untreated wastewater is 5.663%, compared to 4.788% after decolorization, reflecting a slight decrease in toxicity. These findings suggest that C-LV and SMS-PO combination could contribute to sustainable wastewater treatment practices by reducing toxicity in batik industry effluent.

**Keywords:** Batik Wastewater Decolorization, Vannamei Shrimp Chitosan, *P. ostreatus* Baglog Waste, Lethality, Scale structure

## INTRODUCTION

Batik is not only the identity of Indonesia but also a cultural heritage of the country. Batik Lebak Chanting Pradana is a batik industry located in the Lebak region of Banten. The waste from Batik Lebak Chanting Pradana has the potential to pollute the environment. As the production of batik increases, so does the amount of waste generated. If batik wastewater is not properly treated, it can have a negative impact on the environment. Therefore, there is a need for proper and appropriate treatment of batik wastewater, as stated in the Decree of the Governor of DIY No: 281/KPTS/1998. The content of liquid waste from the batik industry can include organic substances, suspended solids, phenol, chromium (Cr), fatty oils, and dyes. Disposing of batik liquid waste into the sewer and eventually into rivers can affect the physical, chemical, and biological conditions. The negative effects of discharging wastewater into rivers include the death of aquatic organisms (Andriani & Hartini, 2017). Carp (*C. carpio*) is a freshwater fish species that is resilient to environmental changes in its surroundings and holds high economic value. It is commonly produced through intensive aquaculture systems (Setiawati et al., 2008). Previous studies have indicated that common carp is often used to test toxicity due to its sensitivity to pollutants in freshwater environments and its ability to react to water physics (Pratiwi et al., 2017).

Decolorization is the process of color destruction or removal of color intensity. Batik industry wastewater contains pollutants, including heavy metals and dyes. Previous studies have indicated that SMSPO has the ability to absorb heavy metals and decolorize batik wastewater effectively (Kartikasari et al., 2012). The disposal of SMS poses environmental toxicity risks, as it releases greenhouse gases through anaerobic digestion, emits foul odors, and produces leachate that can pollute water sources and cause eutrophication. This waste also presents disposal challenges due to its high moisture content and low density (Martín et al., 2023).

CLV is produced from the discarded shells of vannamei shrimp. Vannamei shrimp are typically disregarded and not effectively utilized. Neglecting this waste can result in environmental pollution. The unregulated discharge of shrimp shell waste contributes to environmental toxicity, as it releases pollutants into water sources, leading to disposal issues. Despite some use in animal feed, the vast amount of shrimp shell by-products left unused exacerbates pollution and results in the loss of valuable bioactive materials (Nirmal et al., 2020).

The shells of vannamei shrimp are being utilized to create chitosan products (de Queiroz Antonino et al., 2017). Chitosan is a derivative of chitin, a substance typically present in the exoskeleton of Crustaceans like shrimp, crabs, and lobsters (Iber et al., 2022). Chitosan has been shown to effectively reduce pollutants in wastewater, such as organochloride pesticides, turbidity, suspended solids, oxidized compounds, as well as fat, oil, and textile dye contaminants (Nechita, 2017).

Besides utilizing chitosan derived from Vannamei shrimp, the decolorization process can also involve the utilization of SMSPO. *P. ostreatus* is a white rot fungi species known for its ability to eliminate odors, break down dyes, and absorb heavy metals (Herath et al., 2024). *P. ostreatus* is often present in SMS, which is generated during the process of mushroom cultivation. Unfortunately, this waste is commonly disposed of without proper treatment, resulting in its limited utilization. However, SMSPO still contains valuable components like mushroom mycelium and cellulose. These components exhibit the potential to adsorb heavy metals and colors, making them viable for applications such as metal adsorption and biodecolorization (Kartikasari et al., 2012). As a result, in the cultivation of *P. ostreatus* mushrooms, the utilized growing medium can be appropriately processed instead of being discarded after 4-5 repetitions.

The objective of this study was to explore the effects of batik wastewater decolorization using a combination of CLV and SMSPO on the $LC_{50}$ value of carp and to investigate the impact of that on the scale structure of carp.

## RESEARCH METHODS

### Research Design

The study was conducted as an experimental research using a randomized complete block design (RCBD). The study was carried out at various locations for sampling and testing, including Batik Chanting Pradana in Pancur Village, Bojongleles, Lebak, Banten; Labuan Pandeglang Coastal Fisheries Port; White Oyster Mushroom cultivation in Kadu Dampit Village, Saketi District, Pandeglang, Banten; The Integrated Laboratory of the Faculty of Science, Pharmacy, and Health Mathla'ul Anwar University; the Laboratory of the Faculty of Pharmacy and Science at Universitas Muhammadiyah Prof. Dr. Hamka in Central Jakarta; and the Quality Testing and Application Unit for Fishery Products in Serang.

### Materials and Tools

The research employed various equipment, such as jars, aquariums, aerators, measuring glasses, 50 L plastic jugs, fish nets, analytical scales, pH meters, thermometers, labelling paper, gloves, stationery, microscope slides, surgical tools, and microscopes. The materials used in the study included batik industrial wastewater, decolorized batik industry wastewater (Oktavia et al., 2024), carp, and fish feed.

### Decoloration Process

The samples of carp were collected from a fish farming location in Pandeglang Regency, Banten. The common carp samples used in the study were between 45-60 days old. A total of 36 carp samples were obtained using a filtering tool. Prior to testing, the common carp were acclimated for 7 days in a tank filled with freshwater and aerated using an aerator. Observations were conducted every 24 hours for a period of 7 days. The decolorized industrial batik wastewater (collected from used in totaling 18.000 mL. A mixture of 720 g of CLV and 9.000 g of SMSPO was added to a container containing the 18.000 mL of batik liquid waste. The mixture was then incubated for 96 hours with occasional stirring.

The decolorized industrial batik liquid waste resulting from the combination of CLV and SMSPO was used for the maintenance of common carp. Testing on the common carp was conducted before and after the decolorization of the batik liquid waste. The pre-decolorization and post-decolorization testing of the combination of CLV and SMSPO determined concentrations of 0%, 1,875%, 3,75%, 7,5%, 15%, and 30%. The common carp were maintained in containers or jars filled with the predetermined concentration of the batik wastewater, with the control group containing 0% batik wastewater and only aerated freshwater using an aerator for fish maintenance. This testing was carried out for 96 hours.

### Toxicity Analysis

The lethality test aimed to determine the mortality rate of carp at various concentrations of the decolorized batik wastewater using the combination of CLV and SMSPO. The test used a 96-hour $LC_{50}$. Each container contained well water mixed with the decolorized batik liquid waste at the predetermined concentrations of 0%, 1,875%, 3,75%, 7,5%, 15%, and 30%, with a dilution of 10.000 mL. Observation of the scale structure was performed to compare the scale structure of the common carp before and after the decolorization. The scale structure was observed by taking scales from the fish using tweezers and examining them under a microscope. Environmental parameter measurements were analyzed descriptively, while the common carp lethality data were analyzed statistically using Probit Analysis.

## RESULT AND DISCUSSION

The toxicity test of the decolorized industrial batik liquid waste using the combination of CLV and SMSPO was conducted to determine the toxic concentration that causes lethality to carp fry within a relatively short time. The concentrations used in the toxicity test of the batik wastewater were based on the 96-h $LC_{50}$ and included concentrations of 0%, 1,87%, 3,75%, 7,5%, 15%, and 30%. The determination of this concentration range was set at a lower level than in a prior study, which indicated that goldfish exhibited complete lethality when exposed to batik wastewater at a concentration of 50% (Oktavia & Novi, 2021). To assess the toxic level in a water body, an acute toxicity test is necessary by observing the mortality of test organisms by 50%. The toxicity level of a waste can be determined using test organisms such as freshwater fish because fish are capable of absorbing approximately 75% of the pollutants in the water, while the remaining 25% is absorbed through their food (Mustofa et al., 2018).

In the research process, the industrial batik wastewater obtained a color value of 5.800 Pt-Co before undergoing decolorization, indicating that the color concentration exceeded the threshold limit. Therefore, the decolorization process was carried out with the aim of reducing the color concentration in the industrial batik wastewater to ensure it does not exceed the threshold limit. After the decolorization process, the color value of the industrial batik wastewater was measured at 577 Pt-Co (Oktavia et al., 2024), while the threshold limit for color concentration is 550 Pt-Co (Hussein & Scholz, 2018). Although there was a decrease in the color concentration of the batik wastewater after decolorization, it did not meet the predetermined threshold limit.

The decolorization mechanism of batik industry wastewater using a combination of CLV and SMSPO is attributed to the presence of several components in the SMSPO, such as mycelium and cellulose. These components are responsible for the decolorization of the batik waste. It is known that the mycelium of the fungus can adsorb the dyes present in the batik waste through enzymatic and non-enzymatic processes. The mycelium of the fungus is hydrophobic, while the dyes are hydrophilic, resulting in a hydrophobic-hydrophilic interaction between the fungus mycelium and the dyes. The adsorption process by the fungus mycelium is the initial stage of color change, followed by enzymatic processes (Senthilkumar et al., 2014). The presence of cellulose in the P. ostreatus baglog is considered to play a crucial role in its decolorization capability and can significantly influence the efficiency of batik waste decolorization, resulting in a substantial removal of color. Sawdust containing cellulose can adsorb textile dyes (Daâssi et al., 2016; Rafatullah et al., 2010).

The synthesis of chitosan from chitin involves the breaking of the acetyl groups of chitin and replacing them with amino groups (deacetylation), resulting in the formation of amino groups ($NH_2$) in chitosan. The release of acetyl groups and the presence of amino groups in chitosan give it a positive charge. The presence of amino groups in the carbon chain of chitosan imparts mechanical resistance to the molecule. These amino groups have the ability to bind to colloidal particles, which are generally negatively charged in water. This charge tends to create repulsive forces among particles of the same charge and is the main reason for colloidal stability. Therefore, chitosan, as a coagulant, can destabilize particles in water, causing them to form larger flocs and ultimately settle down (Aulia & Rahayu, 2015).

The data presented in Tables 1, 2, and Figure 1 show that exposure to batik wastewater, both before and after undergoing decolorization, significantly affects the mortality rate of common carp. Before decolorization, wastewater concentrations of 30%, 15%, and 7.5% resulted in a 100% mortality rate. At 3.75%, the mortality rate dropped to 66.7%, and at 1.87%, it further decreased to 38%, while no mortality occurred at 0% concentration. However, following the decolorization process, a marked reduction in mortality was observed; although 30%, 15%, and 7.5% concentrations still caused 100% mortality, no fish mortality occurred at 3.75%, 1.87%, or 0% concentrations.

The high mortality rates at elevated concentrations of untreated batik wastewater indicate that common carp could not withstand the rapid decline in water quality. Fish displayed irregular movements, suggesting disturbances in their equilibrium, with altered behavior manifesting about a week before the average mortality rate was reached. Toxic substances in the wastewater disrupt physiological responses, impacting fish balance and body control (White et al., 2007). Leading up to mortality, carp exhibited behaviors such as loss of balance, gathering near the water's surface, labored breathing, and color fading, all indicative of stress and toxicity (Pratiwi et al., 2016).

**Table 1**

Lethality test results of carp (*C. carpio*) in pre-decolorized batik industrial wastewater

| Concentration (%) | Number of Common Carp | Average lethality of Common Carp at each hour | | | | Number of lethalityper hour | Lethality Percentage (%) |
|---|---|---|---|---|---|---|---|
| | | 24 | 48 | 72 | 96 | | |
| 0 | 18 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1,875 | 18 | 0 | 4 | 2 | 1 | 7 | 38 |
| 3,75 | 18 | 7 | 4 | 1 | 0 | 12 | 66,7 |
| 7,5 | 18 | 16 | 2 | 0 | 0 | 18 | 100 |
| 15 | 18 | 17 | 1 | 0 | 0 | 18 | 100 |
| 30 | 18 | 18 | 0 | 0 | 0 | 18 | 100 |

**Table 2**

Lethality Test Results of Common Carp (*C. carpio*) exposed to post-decolorized batik industrial wastewater

| Concentration (%) | Number of Common Carp | Average lethality of Common Carp at each hour | | | | Number of lethality per hour | Lethality Percentage (%) |
|---|---|---|---|---|---|---|---|
| | | 24 | 48 | 72 | 96 | | |
| 0 | 18 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1,875 | 18 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3,75 | 18 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7,5 | 18 | 0 | 0 | 18 | 0 | 18 | 100 |
| 15 | 18 | 18 | 0 | 0 | 0 | 18 | 100 |
| 30 | 18 | 18 | 0 | 0 | 0 | 18 | 100 |

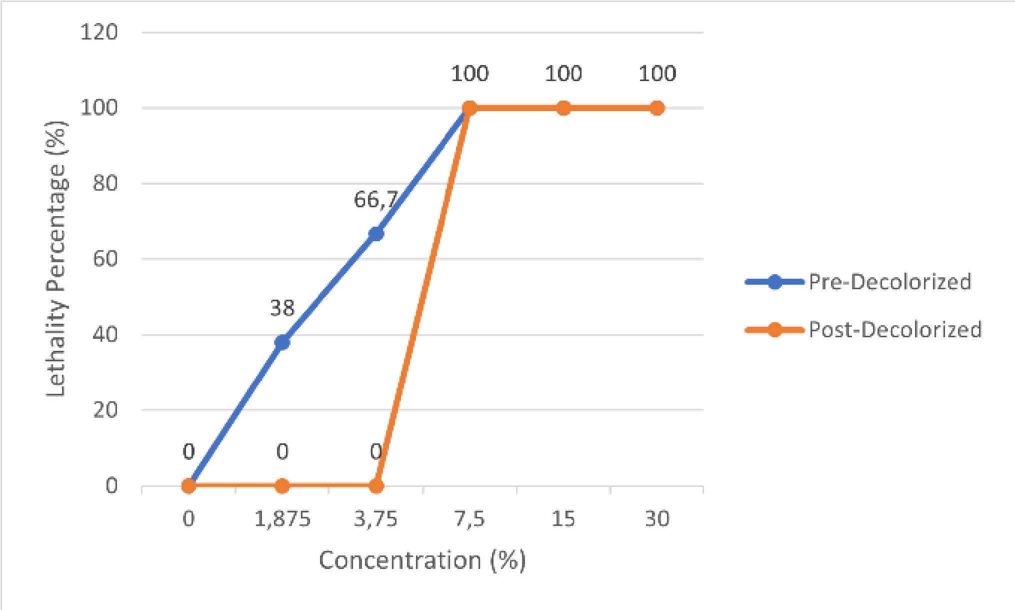
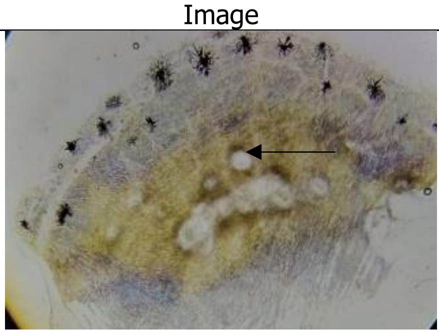
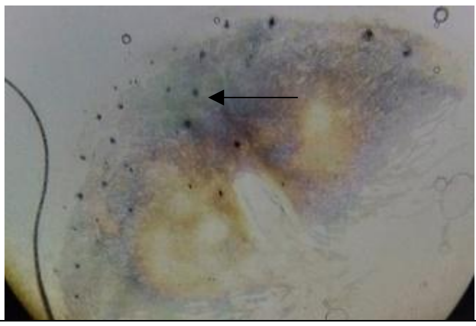



**Figure 1.** Graph of Comparative Lethality Rates in Carp Before and After Decolorization

Based on observations of carp scales exposed to various concentrations of batik industrial wastewater before and after a 96-hour decolorization process under 4x10 microscope magnification (Table 3 and 4), three concentrations—0%, 1.87%, and 3.75%—showed the presence of wastewater residue on the scales, as indicated by arrows in Tables 3 and 4. However, the residue only partially covered the scales. At the 0% concentration, no residue was observed, and the scales appeared clean and undamaged. Conversely, at the 1.87% and 3.75% concentrations, residue was present, though no structural damage to the scales was noted. Andriani & Hartini (2017) similarly reported that external contaminants from batik wastewater adhered to tilapia fry scales at concentrations ranging from 0.37% to 0.73%. As scales are among the outermost organs of fish, they directly reflect pollutant exposure. The amount of contaminant attachment increased in proportion to the toxin concentration, although no changes in the shape or structure of the scales were observed.

**Table 3**

Observation of scales in carp exposed to pre-decolorized batik industrial wastewater for 96 hours using a microscope magnification of 4x10.

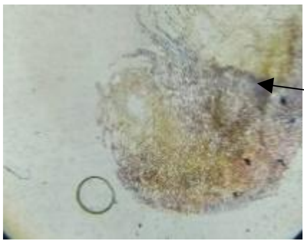
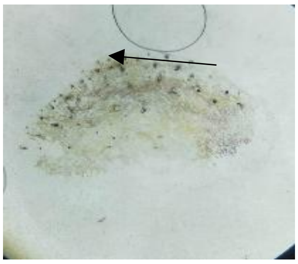
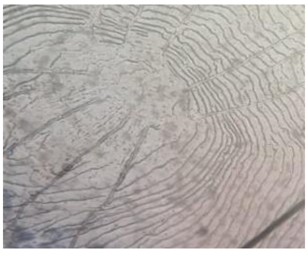
| Concentrations (%) | Image | Description |
|---|---|---|
| 3,75 |  | There is waste adhering to the scales |
| 1,87 |  | There is waste adhering to the scales |

| | | |
|---|---|---|
| 0 |  | There is no waste adhering to the scales |

Decolorization may affect the structure of fish scales by altering their ability to bind with foreign substances, which could influence bioaccumulation patterns. The process potentially reduces or neutralizes certain reactive compounds in the wastewater, decreasing toxic residue attachment to the scales. This suggests that decolorization could lower the bioaccumulation rate of hazardous substances, impacting fish health and influencing the accumulation of toxins in aquatic environments exposed to treated industrial effluents.

**Table 4**

Observation of scale structure in carp exposed to batik industrial wastewater after decolorization for 96 hours using a microscope magnification of 4x10.

| Concentrations (%) | Image | Description |
|---|---|---|
| 3,75 |  | There is waste adhering to the scales |
| 1,87 |  | There is waste adhering to the scales |
| 0 |  | There is no waste adhering to the scales |

## CONCLUSION

Based on the research, it can be concluded that decolorized batik wastewater affects the scale structure of *C. carpio* due to pollutant adhesion. The 96-hour $LC_{50}$ value for batik wastewater before decolorization is 5.663%, and after decolorization, it decreases to 4.788%, showing a reduction in toxicity by 0.875%. These findings suggest that decolorization is an effective method to reduce batik wastewater toxicity, promoting more sustainable waste management in the batik industry. Additionally, combining chitosan and SLS may help reduce effluent toxicity on a larger scale. Further research is needed to optimize this approach and improve wastewater treatment in the batik industry.

## RECOMMENDATION

Future research should focus on studying the long-term effects of decolorized wastewater on *C. carpio* and other aquatic organisms, as well as optimizing the decolorization process to improve efficiency and reduce toxicity. It is also important to compare different decolorizing agents and sources of chitosan to find the most effective methods. Additionally, evaluating the environmental impact and biodegradability of decolorization residues will help assess their safety. Pilot studies should explore the feasibility of large-scale implementation, and alternative wastewater treatment methods should be investigated for agricultural or aquacultural use, ensuring regulatory compliance and safety.

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