

# Mechanisms of Indigosol Blue O4B batik dye wastewater

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**Submission date:** 05-Mar-2023 03:44PM (UTC+0700)

**Submission ID:** 2029113574

**File name:** Mechanisms\_of\_Indigosol\_Blue\_O4B\_batik\_dye\_wastewater.pdf (378.4K)

**Word count:** 5405

**Character count:** 27795

## Mechanisms of Indigosol Blue O4B batik dye wastewater degradation by *Aspergillus* sp. 3 and its product analysis

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### Abstract

The role of fungi in the treatment of dye wastewater has been widely investigated, but not many have reported in full the about the mechanisms and degradation products. The purpose of this study was to determine the mechanism that occurs when microscopic isolate of *Aspergillus* sp. 3 was isolated from the disposal of batik industrial waste in degrading the Indigosol Blue O4B (IB) batik dye wastewater and deciding the degradation products that were formed. The ligninolytic test using tannic acid was carried out for qualitative tests. To prove the mechanism of degradation of IB batik wastewater, the biosorption mechanism assay was carried out (with changes in the color of mycelium formed and measurement of adsorption %) and the biodegradation mechanism assays of both crude and purified enzymes (Lakase, MnP, LiP enzyme).

The products of degradation were identified using UV-Vis spectrophotometer and FTIR absorption measurements. The isolate produced clear zones on agar media with addition of tannic acid. The isolate mechanism was superior in degrading batik IB wastewater by biosorption (adsorption percentage of 33.39%; 50.49%; 68.55% at 24, 48, 72 hours) and enzymatic degradation (laccase enzyme specific activity, MnP, LiP in the amount of 75.087 U/mg; 49.665 U/mg and 129.347 U/mg). The product of degradation was identified as a simple compound which was an aliphatic compound containing C=C, C-O and -OH bonds, so that it was predicted as a group of aliphatic alcohol compounds. This isolate can be used for further development of applications for environmentally friendly dye wastewater processing technology.

**Keywords:** *Aspergillus* sp.3, biosorption, degradation products, ligninolytic.

### Introduction

Indigosol coloring is chosen than others because it has many superior characteristics. These dyes are fast, flat and bright. The dyes can be applied in dyeing and 'coletan' on batik industry<sup>23</sup>. Considering its fastness properties, it is good to

be applied in dyeing. These dyes are characterized by fine dispersion through increasing rapid coloring and are able to be used for high speed in continuous dyeing techniques, brighter colors and better techniques than other dyes during bleaching. Indigosol dyes can be applied in dyeing at various types of fabrics such as cellulose fabric, t-shirts, silk, wool, cotton yarn and also applied to knitted goods<sup>4</sup>.

Indigo dyes are acute toxic, irritating to the skin and eyes, toxic after repeated application, sensitive, mutagenic and carcinogens<sup>17</sup>. Indigosol wastewater is toxic since it contains excess reducing agent sodium hydrosulfite which is oxidized to alkali and alkaline earth metal sulfate ( $\text{SO}_4^{2-}$ ), sulfite ( $\text{SO}_3^{2-}$ ) and thiosulfate ( $\text{S}_2\text{O}_3^{2-}$ ) affecting environmental damage. Wastewater containing sulfate/high sulfite is very corrosive. Thus, the formed sulfate deposits can create toxic hydrogen sulfide (HS) ions<sup>5</sup>. Therefore, easy and inexpensive processing techniques are needed to save the environment.

Fungi isolated from dye industry waste are reported to be able to degrade various coloring wastewater<sup>2,3</sup>. A number of studies have reported that fungi can reduce the concentration of indigo dyes and can degrade indigo dye wastewater.<sup>6,24</sup>

The mechanism of degradation by fungi can be grouped into two processes namely biosorption and/or biodegradation (with ligninolytic enzyme activity). The connection of those two processes in the degradation of indigosol batik wastewater is unknown. Therefore, the decomposition mechanism that occurs in the role of the fungi needs to be investigated. The amount of ligninolytic enzyme activity that plays a role in the mechanism of degradation needs attention. A special discussion on testing specific activities of ligninolytic enzymes is needed to prove that the enzyme is a system with an important role in the process of degradation of batik waste.

Products resulted from degradation of batik waste are important to know. The purpose of this study was to determine the mechanism occurred when microscopic isolate of *Aspergillus* sp. 3 was isolated from the disposal of batik industrial waste in degrading the IB batik dye wastewater and to decide the degradation products been formed.

### Material and Methods

**Materials:** *Aspergillus* sp. 3 was obtained from previous research results. It was isolated from batik wastewater taken

from the disposal location of the batik industry and then selected from others as superior isolate<sup>12,13</sup>. Batik wastewater used in this research was indigosol blue O4B (IB). It was obtained from the batik industry in Banyumas District, Central Java, Indonesia.

## Methods

**1. Preparation of isolate:** Pure culture of *Aspergillus* sp. 3 in the slant media agar was rejuvenated on Potato Dextrose Agar media in Petri dishes, incubated for five days at room temperature and then used at the next stage.

## 2. Determination of the mechanism of degradation of IB batik wastewater by fungi *Aspergillus* sp. 3

**a. Mechanism of biosorption:** The growth medium used in the adsorption mechanism is the Potato Dextrose Broth cultivation medium supplemented with 0.8% diammonium hydrogen phosphate. 75 mL of medium in 250-mL Erlenmeyer flasks were inoculated with  $3.5 \times 10^7$  /mL fungi spores. The inoculated medium was then incubated in a reciprocal shaker at 90 rpm for 96 hours. The fungal mycelium was harvested at the end of incubation by centrifugation using a refrigerated centrifuge and washed with distilled water. Dead biomass was made by autoclaving at 121° C for 15 minutes before harvesting the culture<sup>11</sup>.

The adsorption mechanism step was carried out using 100 mL IB batik dye wastewater in 100-mL Erlenmeyer flask added with 0.25 g biomass fungi dry weight and then incubated for 24 hours above shaker at 90 rpm. After the end of incubation, the biomass was separated by centrifugation and the concentration of dyestuff in the supernatant was measured using spectrophotometry<sup>11</sup>.

$$\% \text{ adsorption} = \frac{C_o - C_e}{C_o} \times 100\%$$

where  $C_o$  = Initial concentration of solution and  $C_e$  = The solution concentration at equilibrium (mg / L).

The adsorption mechanism was known by the color change of the mycelium formed which was originally white to blue like the indigosol blue O4B dye. Color changes were seen as evidenced by microscopic shooting using a SEM microscope. The indication of adsorption is also known by the reduction in solution concentration after the decolorization process.

## b. Mechanism of Biodegradation:

**1) Test enzyme activity:** The fungi of the selected isolates were grown in the PDB medium in the reciprocal shaker with a speed of 90 beats per minute for 96 hours. Mycelium grows to form pellets. The formed pellets are separated from the production medium using centrifuges. Pellets are used as an inoculum to decolorize IB batik wastewater for enzyme production. The supernatant obtained was a crude enzyme and was used to measure enzyme activity after decolorization. Crude enzyme obtained is then used in

measuring enzyme activity. The enzyme activity measured was the enzyme laccase, manganese peroxidase enzyme and lignin peroxidase enzyme activity.

Before the measurement of enzyme activity, crude enzyme production was carried out. The isolate of *Aspergillus* sp. 3 fungal inoculum was used to decolorize wastewater using PDB in room temperature shakers. After 4 days of cultivation, a small pellet is formed, this pellet is homogenized at 1500 rpm for 30 seconds. 8 ml of homogeneous pellet samples were used to inoculate the Erlenmeyer flask. IB batik dye wastewater was then added to the Erlenmeyer Flask. The culture of strain fungi was shaken out in a shaker using an Erlenmeyer flask with agitation of 90 rpm.

The sample was used to test the filtration enzyme activity. The sample was then tested for enzyme activity. The activity was measured by the enzyme is LiP, MnP, Lac.

Laccase activity was obtained by mixing 900 µl of filtrate culture with 750 µl of sodium acetate buffer 0.1 M and then added with 100 µl of syringaldazine in 0.5 mM at room temperature. Then the absorbance measurements were carried out at 525 nm. The absorbance reading for 1 minute was repeated several times until the data is good.

MnP activity was obtained by mixing 1000 µl of the filtrate culture with 1750 µl malanoic acid mM pH 4.5 and then adding 125 µl 2.6 DMP 20 mM and 125 µl manganese sulfate ( $\text{MnSO}_4$ ) 20 mM and then adding also with 300 µl of 2 mM  $\text{H}_2\text{O}_2$ . Subsequent absorbance measurements were carried out at 470 nm. The absorbance reading for 1 minute was repeated several times until the data is good.

The LiP activity tested was obtained by mixing with 1000 µl of filtrate culture and 300 µl of 2mM  $\text{H}_2\text{O}_2$ , then adding 2000 µl of LiP buffer (Tween 80 0.5 g, veratyl alcohol 33.6 mg and 0.1 M buffer sodium tartrate solution 500 mL pH 2.9). Then the absorbance measurements were carried out at 310 nm. The absorbance reading for 1 minute was repeated several times until the data was good.

**2) Enzyme Purification:** Purification of the enzyme is called enzyme purification. This stage is through deposition of ammonium sulfate<sup>22</sup>, dialysis<sup>22</sup> and gel filtration column chromatography.

Crude enzyme was produced then to be deposited using ammonium sulfate salt to obtain saturation with concentrations of 0-20%, 20-40%, 40-60% and 60-80%. Salt was added slowly in cold conditions by continuing to stir. The mixture obtained was then centrifuged for 20 minutes at a speed of 3580 g. Protein deposits were dissolved with 0.5 M pH acetate buffer 5. The deposition fraction of ammonium sulfate at each concentration was then tested for laccase enzyme activity, MnP and LiP and protein content to determine the best ammonium sulfate fraction.



Dialysis was carried out by cutting the dialysis membrane with 10 kD and washing it in running water<sup>22</sup>. The dialysis membrane was boiled in a mixture of 2% (b/v) Na-Carbonate and 0.05% (b/v) EDTA for 10 minutes. Dialysis membranes were boiled again in distilled water twice for 10 minutes each. The dialysis membrane was cooled and fastened to one end of the membrane. The fractionated ammonium sulfate extract with the highest specific activity was pipetted into the membrane.

The membrane that has been fastened was immersed in acetate buffer 0.5 M, pH 5 with a ratio of 100 times the sample volume. The dialysis process was carried out at 4° C with stirring for 9 hours with three times the replacement of the soaking buffer. Dialysis enzymes were tested for laccase, MnP and LiP activity and protein content.

The dialysis results obtained were then purified using gel filtration column chromatography<sup>18</sup>. The Sephadex G100 gel matrix in the column is slowly flown with acetate buffer 0.5 M pH 5 for rinsing. The dialysis enzyme extract was passed into a column containing Sephadex G100 and eluted with an acetate buffer 0.5 M pH 5 with a flow rate of 1.66 x 10<sup>-5</sup> L. s<sup>-1</sup>. Each mL of the fraction formed was analyzed for the activity of lakase enzymes, MnP and LiP and their protein content.

**3) Determination of specific activities of purified enzymes:** Specific activity test aims to determine the size of laccase, MnP and LiP produced by isolate of *Aspergillus* sp. 3 fungi. The results are expressed by U/mg which proves that the *Aspergillus* sp. 3 produces an enzyme of a number of values obtained. These results also assume the extent of the role of the three enzymes in decolorizing IB batik wastewater.

Protein values were calculated using the Bradford method<sup>7</sup>. The test solution (enzyme fraction) of 5 µL was mixed with 100 µL of Bradford solution in cuvette. The mixture is homogenized for 10 seconds. The mixture was left to stand for 10 minutes and then measure its absorbance at a wavelength of 595 nm. The standard used is bovine serum albumin (BSA) with a concentration of 0-100 ppm.

**3. Product Analysis results from degradation of IB batik wastewater by *Aspergillus* sp. 3 fungi:** *Aspergillus* sp. 3 fungi are grown at optimum conditions. After incubation, filtration is then carried out. The solution obtained was accommodated in a sterile Erlenmeyer flask and then used for identification using a UV-Vis spectrophotometer<sup>8</sup> and FTIR<sup>19</sup>.

Identification of the products resulting from degradation using a UV-Vis spectrophotometer was carried out to see changes in the spectrum. Measure absorbance with a UV-Vis spectrophotometer at one minute intervals for 30 minutes. Absorption of the spectrum between 275 and 800 nm is recorded during decolorization.

FTIR identification is used to study changes in dye structure before and after degradation. The 50 ml solution of the degradation solution sample was characterized using a Fourier Transform Infrared Spectrometry (FTIR) spectrometer. The wavelength range used was 400-4600 / cm.

## Results and Discussion

### 1. Determination of the degradation mechanism of IB batik wastewater by fungi *Aspergillus* sp. 3:

**a. Mechanism of biosorption:** The biomass of fungi was used to study their capacity to adsorb dye from its solution<sup>11</sup>. The presence of dead mycelium was used to prove the existence of decolorization mechanism through adsorption because with the death of the cell, there was no enzyme involvement in decolorization so that only adsorption event occurred.

The pellet surface observations of *Aspergillus* sp. 3 were incubated within 24, 48 and 72 hours and presented in figure 1. Pellets appear to be colored blue when compared to control isolates without the addition of IB batik wastewater. Since the incubation of the first day, the blue color has begun to stick both of pellet surface and cell wall. This proves that the adsorption mechanism occurs during incubation.

Evidence of adsorption was also shown in observations using SEM. SEM microscopy has been used as a tool for biosorbent characterization<sup>21</sup> because it can show the accumulation of dyes on the surface<sup>11</sup>. The optical microscopic image of *Aspergillus* sp. 3 was shown in figure 2. The SEM image supports the proof of the adsorption of IB batik wastewater.

Before the treatment, it showed the morphology of mycelium surface which looked smooth; no particles were stuck and irregular with a large area for dye surface interactions. Significant changes occurred in the morphology of mycelium surface and became compact after adsorption of IB batik wastewater. SEM images showed colored molecules. IB batik wastewater was adsorbed in a completed form. The surface of the mycelium looked rough and there were particles stuck.

Proving the existence of an adsorption mechanism that occurs, out further treatment was carried out by calculating the percentage of dead mycelium adsorption so as not to be affected by cell metabolic activity. The results of the percentage of adsorption obtained were presented in figure 4. The percentage of adsorption increased with incubation time. The average value obtained was 33.39% on the first day, then 50.49% on the second day and 68.55% on the 3rd day.

The observation of dead mycelium surface was also done to ensure the mechanism occurred. Microscopic appearance of dead mycelium after decolorization could be seen in figure 4.

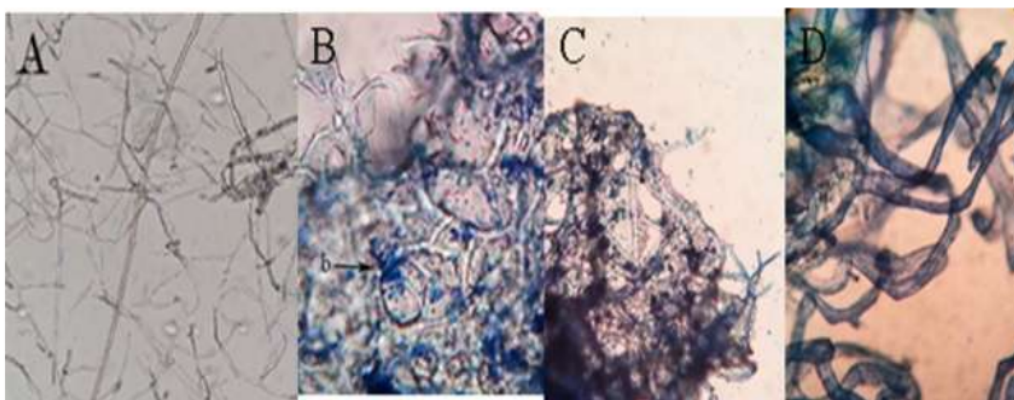


Figure 1: Pellet surface of *Aspergillus* sp. 3 on mycelium without treatment of (A) IB batik wastewater, (B) 24 hours, (C) 48 hours and (D) 72 hours incubation time.

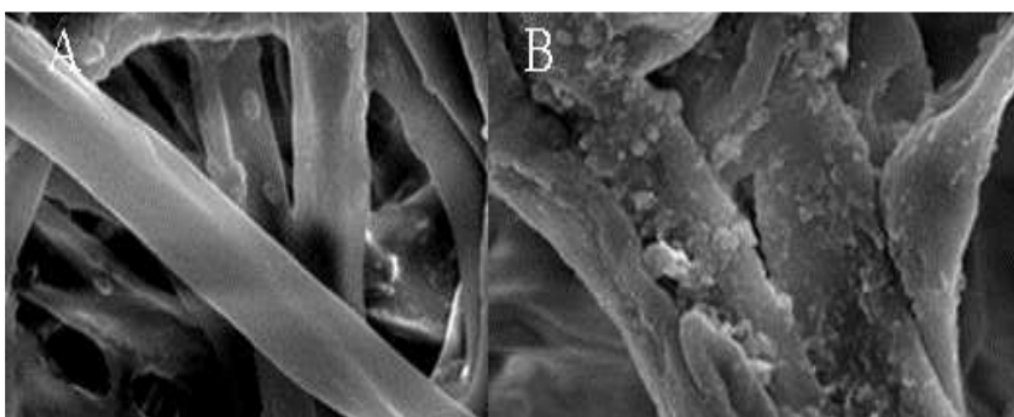


Figure 2: SEM of *Aspergillus* sp. 3. Isolate of *Aspergillus* sp. (A) 3 before and (B) after decolorization treatment at magnification 7500x.

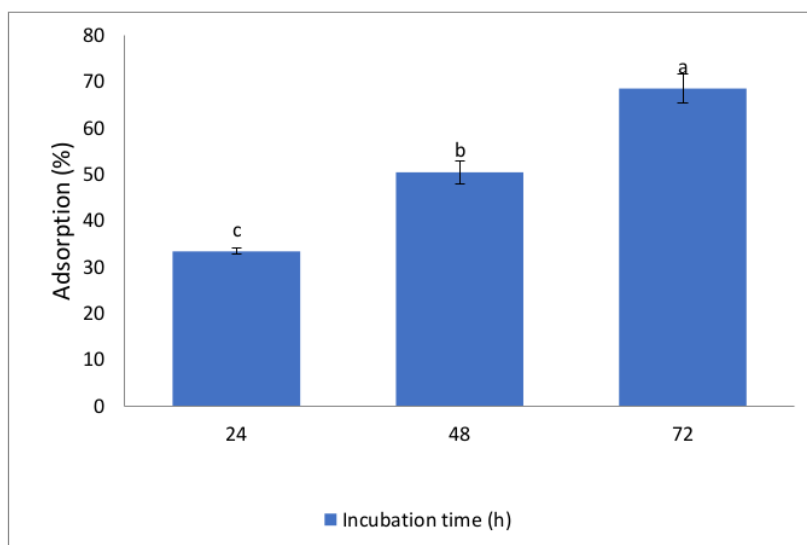


Figure 3: Percentage of *Aspergillus* sp. 3 adsorption at dead mycelium.



**Figure 4: Dead mycelium after decolorization. The dye was adsorbed on (a) the cell surface, the dye accumulates in (b) the cytoplasm.**

The image provides information telling about the dead mycelium with presence of dyes that stick to the surface of the mycelium and also the presence of dyes entering the cytoplasm. This indicated that the mechanism occurred during degradation was a biosorption mechanism either adsorption or intracellular accumulation. Biosorption can be classified as adsorption/cell surface precipitation and intracellular accumulation depending on location of adsorbate<sup>15</sup>. The adsorption surface is generally assisted through ionic, chemical and physical interactions.

Figure 4 showed the mycelium after adsorption process. This showed that the dye molecule was adsorbed on the cell surface. The cell wall of fungi contains large sources of chitin, chitosan, 1,3-D-glucan, α-1,6-D-glucan and mannoprotein on different functional groups such as carboxyl, amine, hydroxyl, phosphate and sulfonate<sup>11</sup>. The presence of amino groups, carboxyl, hydroxyl, phosphate and sulfonates in biomass can interact with dyes. Therefore, the initial attachment to the dye molecule with the cell surface follows a complex pattern.

The dye adsorption of batik IB wastewater on biomass occurs through (1) chemical interactions between colored molecules and fungi cell wall components, (2) electrostatic interactions between dye molecules and electron-rich sites on the cell surface and (3) weak physical forces such as hydrogen bonds and van der Waals. Cell walls interact between

hydrophobic parts of colored molecules (for example, aromatic rings) and biomass polysaccharides.

During the incubation in this study, it can be observed that the color of IB batik wastewater changes from blue to a clear color. It relates to previous research that the color of the solution changes from blue to light blue to a clear color, as in controls without dyes because of the activity of laccase enzymes that is able to oxidize indigo dyes<sup>24</sup>.

The mechanism of biodegradation is a breakdown of dyes which involve activity of various enzymes<sup>1</sup>. Measurements of enzyme activity in this study were carried out to prove the mechanism of biodegradation and to know the enzymes which play a role in the decolorization of IB batik wastewater. It is known that fungal enzymes such as laccase (Lac), manganese peroxidase (MnP), lignin peroxidase (LiP) play a role to degrade dyes<sup>20</sup>. The activity of LiP, MnP and Lac enzymes assayed on crude enzyme can be seen in tables 1, 2 and 3.

Based on table 1, it can be seen that laccase activity in crude enzyme was 11,666.67 (U) where the specific activity was 58.39 (U/mg). Table 2 informed that the magnitude of MnP enzyme activity was 5,256.048 (U) with a specific activity of 26,307 (U/mg) and table 3 showed for LiP has an enzyme activity of 156,838.7(U) with specific activity 784,98 (U / mg).



Test results of specific activities of laccase, MnP and LiP enzymes were presented in table 1, 2 and 3. The data in the table proved that there was an increase in activity after enzyme purification from dialysis level to chromatographic column. The laccase enzyme specific activity increased from 58.39 to 148.39 and then to 1091.02 U/mg. Similarly, the peroxidase enzyme increased from 26.31 to 26.44 and 198.66 U/mg for MnP and 784.98 to 3682.4 and 5638.21 U/mg for LiP.

## 2. Product Analysis results from degradation of IB batik wastewater by *Aspergillus sp. 3* fungi:

**UV-VIS:** The analysis of products from degradation using a UV-Vis spectrophotometer was carried out to see changes in the maximum absorption spectrum. Changes in peak spectrum occurred during decolorization. The UV-Vis spectrophotometer showed a sharp new peak at different wavelengths than the wavelength before the degradation process.

Figure 5 presented graphical form at the visible wavelength. The results of UV-Vis analysis of IB batik wastewater samples before decolorization showed a peak detected at a wavelength of 604.5 nm (Figure 5a). The curve in figure 5a showed the maximum wavelength seen in IB batik

wastewater but was not seen in the decolorization results. The peaks seen in IB batik wastewater were not seen in decolorization results with isolate of *Aspergillus sp. 3*. Figure 5b showed that the decolorization solution showing a peak that cannot be detected at the visual wavelength (400-750 nm). The peak was in the UV wave at a wavelength of 345 nm. This showed that there was a degradation characterized by the loss of peaks and the existence of other compound with the appearance of new peak.

Figure 5 indicated the degraded IB batik wastewater sample showing undetectable peaks at wavelengths of 400 and 800 nm. This proved that after the decolorization assay, there was a reading shift in the wavelength. The occurrence of a wavelength shift also showed degradation. This shift caused the structure of the IB batik wastewater changed and conjugated bond decreased, resulting in a shift in the wavelength toward a shorter wavelength<sup>25</sup>.

**FTIR:** FTIR was used to characterize and identify functional groups of IB batik wastewater before and after treatment using isolate of *Aspergillus sp. 3*. The FTIR spectrum in both samples was measured on absorption bands at 400-4000 cm<sup>-1</sup>.

Table 1  
Stages of laccase enzyme purification from superior fungi isolates

Stage	Volume (ml)	enzyme Activity (U)	Protein (mg/ml)	total protein (mg)	Specific Activity (U/ mg)	(%)	Purification factor
Crude enzyme	1000	11,666.67	0.19	199.8	58.39	100	1
Dialysis	25	1,316.24	0.36	8.87	148.39	11.28	2.54
Kromatografi kolom	7.5	255.77	0.01	0.23	1,091.02	2.19	18.68

Table 2  
Stages of MnP enzyme purification from superior fungi isolates

Stage	Volume (ml)	enzyme Activity (U)	Protein (mg/ml)	Total protein (mg)	Specific Activity (U/ mg)	(%)	Purification factor
Crude enzyme	1000	5,256.05	0.199	199.8	26.31	100	1
Dialysis	25	234.57	0.355	8.87	26.44	4.46	1.01
Kromatografi kolom	7.5	13.97	0.009	0.07	198.66	0.27	7.55

Table 3  
Stages of LiP enzyme purification from superior fungi isolates

Stage	Volume (ml)	enzyme Activity (U)	Protein (mg/ml)	Total protein (mg)	Specific Activity (U/ mg)	(%)	Purification factor
Crude enzyme	1000	156,838.70	0.19	199.8	784.98	100	1
Dialysis	25	32,662.90	0.36	8.87	3,682.40	20.83	4.69
Kromatografi kolom	7.5	396.53	0.01	0.07	5,638.21	0.25	7.18

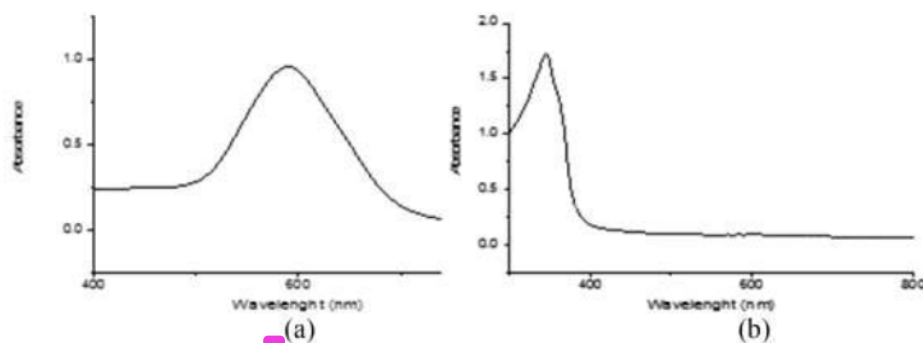


Figure 5: Spectra UV-Vis of IB batik wastewater (a) before and (b) after treatment using *Aspergillus* sp. 3.

Table 4  
Spectra of FTIR IB batik wastewater before and after degradation using isolate of *Aspergillus* sp.3

Sample	Number of Waves (cm <sup>-1</sup> )	Functional groups
IB batik wastewater	501.49, 555.50, 594.08, 617.22	C-Br Association
	1519.91, 1543.05, 1558.48	Aromatic and aliphatic groups (stretch C = C) and C = N-
	1697.36	Cluster N-H
	3734.19	Hydroxyl group (O-H).
Degradation products	1635.67	Aliphatic C=C
	2108.38	C-O stretch group
	3332.05	-OH stretch

Figure 6 and table 4 showed FTIR spectra of IB batik wastewater before and after degradation. FTIR spectra data in figure 6a for IB batik wastewater showed the presence of several absorption bands. Absorption bands that appear in IB batik wastewater samples were 501.49; 555.50; 594.08; 617.22, 1519.91; 1543.05; 1558.48, 1697.36, 3734.19 cm<sup>-1</sup>.

Absorption band at wave number 501.49; 555.50; 594.08; 617.22 cm<sup>-1</sup> indicates the presence of C-Br bonds. As also reported by researchers, alkyl halide is recognized by the appearance of absorption bands in the region of 200-750 cm<sup>-1</sup>, especially in the area of 500-680 cm<sup>-1</sup> for C-Br<sup>23-26</sup>.

The analysis results in figure 6a also show the absorption bands of aromatic and aliphatic groups (strain C=C) and C=N- appearing at wave number 1519.91, 1543.05, 1558.48 cm<sup>-1</sup>. According to Clifford et al<sup>10</sup>, at wave number 1675-1500 cm<sup>-1</sup> there is a C=C (aliphatic and aromatic) strain and a C=N- strain. The peak caused by stretching vibrations of C=C) and C=N- was located in the wave number 1690-1600 cm<sup>-1</sup>. The aromatic ring shows peaks in the region of 1650-1450 cm<sup>-1</sup> and with a low degree of substitution shows the peaks at 1600, 1580, 1500 and 1450 cm<sup>-1</sup> 9,16,26,27,29.

The wave number of 1697.36 cm<sup>-1</sup> showed the absorption of the N-H group which was a special structure of Indigosol. The absorption band at 3734.19 cm<sup>-1</sup> showed the presence of the hydroxyl group (O-H). Amide bonds to a number of waves 1670-1700 cm<sup>-1</sup> and the wavenumber 3750 - 3000 cm<sup>-1</sup> show a stretch of -OH.<sup>9,14,16, 26-29</sup>

FTIR spectra data from degradation products which are IB batik waste after treatment using *Aspergillus* sp.3 were presented in figure 6b. The absorption bands that emerged from the degradation product were different than before treatment. These results indicated the loss of the functional groups of the dye structure. The degradation products showed that there were only three absorption bands remaining namely 1635.67, 2108.38 and 3332.05 cm<sup>-1</sup>.

Aliphatic group or strain C=C is located in the frequency of the wave number 1675-1500 cm<sup>-1</sup> 10. Wave number 2108.38 cm<sup>-1</sup> that appears with an intensity of 95.931% was a C-O stretching group. The C-O stretching group at the wave number 2108 cm<sup>-1</sup> 30. -OH stretching appeared to be read in the region of wave number 3332.05 cm<sup>-1</sup> with an intensity of 45.806%. -OH stretching at absorption from 3750-3000 cm<sup>-1</sup> 10.

Based on the results of the FTIR analysis, it can be assumed that in the indigosol blue O4B colorant contained in the IB wastewater, there were aliphatic, aromatic and C-Br alkyl halide groups and NH groups while after the treatment there are only three functional groups namely C=C, C-O groups and -OH while the typical Indigosol groups namely aromatic, NH and Br did not appear. This indicated that there was a degradation process by *Aspergillus* sp. 3. This isolate breaks the aromatic bond, C-Br alkyl halogen and -NH into simpler compounds. The compound has a bond from the remaining three groups which are predicted as alkenes with hydroxyl groups to become alkenol (CH<sub>4</sub>=C-OH).



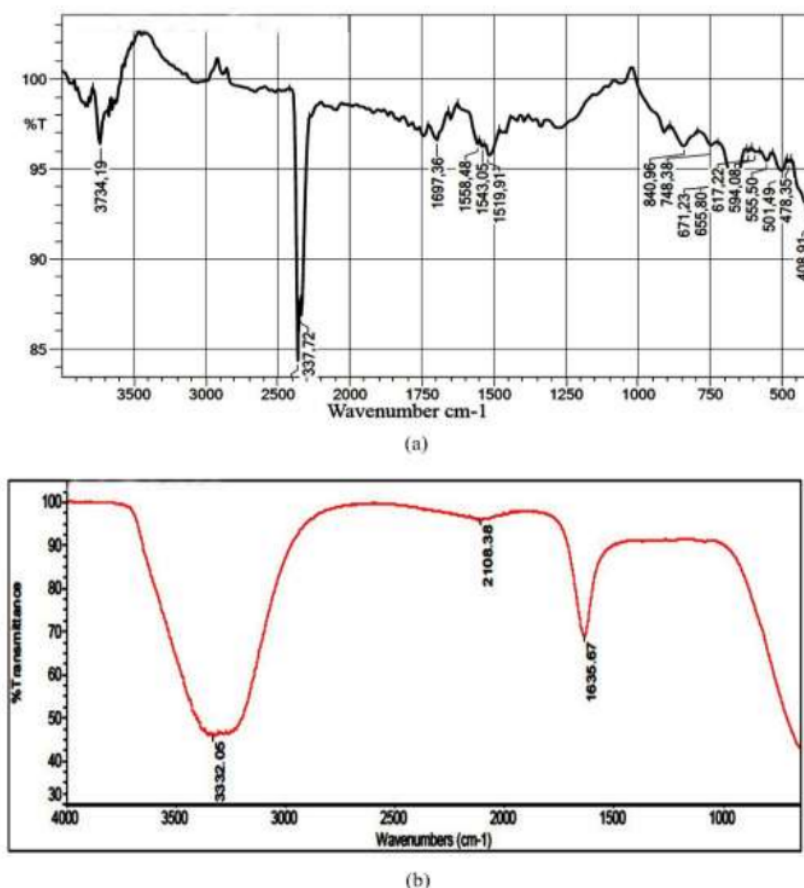


Figure 6: FTIR spectrum (a) before degradation and (b) degradation products IB batik wastewater using isolate of *Aspergillus sp. 3*

## Conclusion

The mechanism of isolate of *Aspergillus sp. 3* in degrading IB batik wastewater was by using biosorption (adsorption) and biodegradation (enzymatic). Decolorization value with the mechanism of degradation through adsorption at 24, 48, 72 hours was 33, 39%; 50.49%; and 68.55%. Extracellular enzymes that were predominantly involved in degradation were LiP, MnP and laccase.

Purified enzymes on column chromatography from isolate had specific activities of laccase, MnP, LiP up to 1,091.02, 198.66 and 5,638.21 U / mg. The product of degradation was identified as a simple compound named aliphatic compound containing C=C, C-O and -OH bonds predicted as aliphatic alcohol compound group.

## Acknowledgement

This research was funded by Directorate of Research and Community Service, Directorate General of Research and Development Strengthening with the Doctoral Dissertation Research scheme.

## References

1. Aksu Z. and Donmez G.A., Comparative study on the biosorption characteristics of some yeasts for Remazol Blue reactive dye, *Chemosphere*, **50**, 1075–1083 (2003)
2. Ali N., Hameed A., Ahmed S. and Khan A.G., Decolorization of structurally different textile dyes by *Aspergillus niger* SA1, *World J Microbiol Biotechnol*, **24**, 1067–1072 (2008)
3. Ali N., Hameed A., Siddiqui M.F., Ghumro P.B. and Ahmed S., Application of *Aspergillus niger* SA1 for the enhanced bioremoval of azo dyes in Simulated Textile Effluent, *African Journal of Biotechnology*, **8**(16), 3839–3845 (2009)
4. Anonim, Indigo Sol Vat, <http://www.jagson.com/indigo-sol-vat.php>, Accessed 5/27/2016 (2011)
5. Blackburn R.S., Bechtold T. and John P., The development of indigo reduction methods and pre-reduced indigo products, *Coloration Technology*, **125**(4), 193–207 (2009)
6. Balan D.S. and Monteiro R.T., Decolorization of textile indigo dye by ligninolytic fungi, *J Biotechnol*, **89**, 141–145 (2001)

7. Bradford M.M., A rapid and sensitive method for the quantitation of microorganisms quantities of protein in utilizing the principle of proteindye binding, *Anal. Biochem*, **72**, 248-254 (1976)
8. Camarero S., Ibarra D., Martí'nez M.J. and Martí'nez A.T., Lignin-derived compounds as efficient laccase mediators for decolorization of different types of recalcitrant dyes, *Appl. Environ. Microbiol.*, **71**(4), 1775–1784 (2005)
9. Christian G.D., Analytical Chemistry, 2<sup>nd</sup> edition, John Wileys & Sons, New York (2014)
10. Clifford J.C., Olaf A.R. and Campbell M., Analisis Spektrum Senyawa Organik, Translated by Kosasih Padmawinata, ITB, Bandung (1982)
11. Das S.K., Bhowal J., Das A.R. and Guha A.K., Adsorption behavior of Rhodamine B on *Rhizopus oryzae* biomass, *Langmuir*, **22**, 7265-7272 (2006)
12. Dewi R.S., Kasiandari R.S., Martani E. and Purwestri Y.A., Bioremediation of Indigosol Blue 04B batik effluent by indigenous fungal isolates, *Aspergillus* spp, *Omni-Akuatika*, **14**(2), 11-20 (2018a)
13. Dewi R.S., Kasiandari R.S., Martani E. and Purwestri Y.A., Decolorization and detoxification of batik dye effluent containing Indigosol Blue-04B using fungi isolated from contaminated dye effluent, *Indonesian Journal of Biotechnology*, **23**(2), 54-60 (2018b)
14. Fatriasari W., Syafii W., Wistara N., Syamsul K. and Prasetya B., Lignin and cellulose changes of betung bamboo (*Dendrocalamus asper*) pretreated microwave heating, *International Journal on Advanced Science Engineering Information Technology*, **6**(2), 186-195 (2016)
15. Gadd G.M., Accumulation of metals by microorganisms and algae, *Biotechnology, Special Microbial Processes*, **6b**, 401-433 (1988)
16. Hargis L.G., Analytical chemistry: Principles and techniques, Prentice Hall Inc., New Jersey (1988)
17. Hunger K., Industrial Dyes Chemistry, Properties, Applications. Wiley-VCH Verlag GmbH & Co. KGaA, Darmstadt (2003)
18. Irshad M., Asgher M., Sheikh M.A. and Nawaz H., Purification and characterization of laccase produced by *Schizophyllum commune* IBL-06 in solid state culture of banana stalks, *Bio Resources*, **6**(3), 2861-2873 (2011)
19. Kariyajjanavar P., Narayana J. and Nayaka Y.A., Electrochemical degradation of C.I. Vat Orange 2 Dye on carbon electrode, *Inventi Impact: Water & Environment*, **3**, 106-112 (2013)
20. Kaushik P. and Malik A., Fungal dye decolourization: Recent advances and future potential, *Environment International*, **35**(1), 127-141 (2009)
21. Mogollon L., Rodriguez R., Larrota W., Ramirez N. and Torres R., Biosorption of nickel using filamentous fungi, *Appl. Biochem. Biotechnol.*, **70-72**, 593-60 (1998)
22. Patel H., Gupte S., Gahlout M. and Gupte A., Purification and characterization of an extracellular laccase from solid-state culture of *Pleurotus ostreatus* HP-1, *3 Biotech.*, **4**, 77-84 (2014)
23. Susanto S.K.S., Seni Kerajinan Batik Indonesia, Balai Penelitian Batik dan Kerajinan, Lembaga Penelitian dan Pendidikan Industri, Departemen Perindustrian, Jakarta (1973)
24. Tian C.E., Tian R., Zhou Y., Chen Q. and Cheng H., Decolorization of indigo dye and indigo dye-containing textile effluent by *Ganoderma weberianum*, *African Journal of Microbiology Research*, **7**(11), 941-947 (2013)
25. Widihati I.A.G., Diantariani N.P. and Nikmah Y.F.T., Fotodegradasi metilen biru dengan sinar uv dan katalis  $Al_2O_3$ , *Jurnal Kimia*, **5**(1), 31-42 (2011)
26. Pecsok R.L., Shields L.D., Cairns T. and McWilliam I.G., Modern methods of chemical analysis, Wiley, New York (1968)
27. Khopkar S.M., Konsep Dasar Kimia Analitik, UI-Prees, Jakarta (1990)
28. Kumar A., Negi Y.S., Choudhary V. and Bhardwaj N.K., Characterization of Cellulose Nanocrystals Produced by Acid-Hydrolysis from Sugarcane Bagasse as Agro-Waste, *Journal of Materials Physics and Chemistry*, **2**(1), 1-8 (2014)
29. Nurdin D., Eludasi Struktur Senyawa Organik, Angkasa, Bandung (1986)
30. Venkov T.V., Hess C. and Jentoft F.C., Redox properties of vanadium ions in SBA-15-supported vanadium oxide: An FTIR spectroscopic study, *Langmuir*, **23**(4), 1768-1777 (2007).

(Received 03<sup>rd</sup> August 2020, accepted 05<sup>th</sup> October 2020)

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